

SEQUENCE LISTING

<110> Terumo Corporation

<120> Functional Hybrid Polypeptide with Collagen-binding
Activity

<130> 19990120

<140>

<141>

<160> 16

<170> PatentIn Ver. 2.0

<210> 1

<211> 343

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Modified Human
Fibronectin Collagen-Binding Domain

<220>

<221> INIT __MET

<222> (1)

<220>

<221> DOMAIN

<222> (2)..(341)

<223> /note="human fibronectin collagen-binding domain"

<220>

<221> CONFLICT

<222> (69)

<220>

<221> CONFLICT

<222> (125)

<400> 1

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 Gly His Cys Val Thr Asp Ser Gly Val Val Tyr Ser Val Gly Met Gln
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 Trp Leu Lys Thr Gln Gly Asn Lys Gln Met Leu Cys Thr Cys Leu Gly
 35 40 45
 Asn Gly Val Ser Cys Gln Glu Thr Ala Val Thr Gln Thr Tyr Gly Gly
 50 55 60
 Asn Ser Asn Gly Glu Pro Cys Val Leu Pro Phe Thr Tyr Asn Gly Arg
 65 70 75 80
 Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg Gln Asp Gly His Leu Trp
 85 90 95
 Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp Gln Lys Tyr Ser Phe Cys
 100 105 110
 Thr Asp His Thr Val Leu Val Gln Thr Arg Gly Gly Asn Ser Asn Gly
 115 120 125
 Ala Leu Cys His Phe Pro Phe Leu Tyr Asn Asn His Asn Tyr Thr Asp
 130 135 140
 Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys Gly Thr Thr
 145 150 155 160
 Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly Phe Cys Pro Met Ala Ala
 165 170 175
 His Glu Glu Ile Cys Thr Thr Asn Glu Gly Val Met Tyr Arg Ile Gly
 180 185 190

Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met Arg Cys Thr
 195 200 205
 Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Ile Ala Tyr Ser Gln
 210 215 220
 Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn Asp
 225 230 235 240
 Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr Cys
 245 250 255
 Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val Asp Gln Cys
 260 265 270
 Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp Ser Trp Glu
 275 280 285
 Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr Gly Arg Gly
 290 295 300
 Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser Ser
 305 310 315 320
 Gly Pro Val Glu Val Phe Ile Thr Glu Thr Pro Ser Gln Pro Asn Ser
 325 330 335
 His Pro Ile Gln Trp Leu Glu
 340

<210> 2

<211> 159

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Human Basic
 Fibroblast Growth Factor with Enterokinase

Recognition Sequence

<220>

<221> PEPTIDE

<222> (1).. (5)

<223> /note="enterokinase recognition sequence"

<220>

<221> PEPTIDE

<222> (6).. (159)

<223> /note="human fibroblast growth factor"

<400> 2

Asp Asp Asp Asp Lys Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu

1 5 10 15

Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp

20 25 30

Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His

35 40 45

Pro Asp Gly Arg Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile

50 55 60

Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly

65 70 75 80

Val Cys Ala Asn Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu

85 90 95

Ala Ser Lys Cys Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu

100 105 110

Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr

115 120 125

Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly

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      130              135              140
Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
145              150              155

<210> 3
<211> 58
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:Human Epidermal
      Growth Factor with Enterokinase Recognition
      Sequence
<220>
<221> PEPTIDE
<222> (1)..(5)
<223> /note="enterokinase recognition sequence"
<220>
<221> PEPTIDE
<222> (6)..(58)
<223> /note="human epidermal growth factor"
<400> 3

Asp Asp Asp Asp Lys Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp
   1               5               10             15
Gly Tyr Cys Leu His Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp
           20                25                30
Lys Tyr Ala Cys Asn Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln
       35                 40                 45
Tyr Arg Asp Leu Lys Trp Trp Glu Leu Arg

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50

55

<210> 4

<211> 501

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Hybrid

Polypeptide of Human Fibronectin Collagen-Binding

Domain and Human Basic Fibroblast Growth Factor

<220>

<221> INIT __MET

<222> (1)

<220>

<221> DOMAIN

<222> (2)..(341)

<223> /note="human fibronectin collagen-binding domain"

<220>

<221> PEPTIDE

<222> (343)..(347)

<223> /note="enterokinase recognition sequence"

<220>

<221> PEPTIDE

<222> (348)..(501)

<223> /note="human fibroblast growth factor"

<400> 4

Met Ala Ala Val Tyr Gln Pro Gln Pro His Pro Gln Pro Pro Pro Tyr

1

5

10

15

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 20 25 30
 Trp Leu Lys Thr Gln Gly Asn Lys Gln Met Leu Cys Thr Cys Leu Gly
 35 40 45
 Asn Gly Val Ser Cys Gln Glu Thr Ala Val Thr Gln Thr Tyr Gly Gly
 50 55 60
 Asn Ser Asn Gly Glu Pro Cys Val Leu Pro Phe Thr Tyr Asn Gly Arg
 65 70 75 80
 Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg Gln Asp Gly His Leu Trp
 85 90 95
 Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp Gln Lys Tyr Ser Phe Cys
 100 105 110
 Thr Asp His Thr Val Leu Val Gln Thr Arg Gly Gly Asn Ser Asn Gly
 115 120 125
 Ala Leu Cys His Phe Pro Phe Leu Tyr Asn Asn His Asn Tyr Thr Asp
 130 135 140
 Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys Gly Thr Thr
 145 150 155 160
 Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly Phe Cys Pro Met Ala Ala
 165 170 175
 His Glu Glu Ile Cys Thr Thr Asn Glu Gly Val Met Tyr Arg Ile Gly
 180 185 190
 Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met Arg Cys Thr
 195 200 205
 Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Ile Ala Tyr Ser Gln
 210 215 220
 Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn Asp

225					230					235					240
Thr	Phe	His	Lys	Arg	His	Glu	Glu	Gly	His	Met	Leu	Asn	Cys	Thr	Cys
				245					250					255	
Phe	Gly	Gln	Gly	Arg	Gly	Arg	Trp	Lys	Cys	Asp	Pro	Val	Asp	Gln	Cys
			260					265					270		
Gln	Asp	Ser	Glu	Thr	Gly	Thr	Phe	Tyr	Gln	Ile	Gly	Asp	Ser	Trp	Glu
		275					280					285			
Lys	Tyr	Val	His	Gly	Val	Arg	Tyr	Gln	Cys	Tyr	Cys	Tyr	Gly	Arg	Gly
	290					295					300				
Ile	Gly	Glu	Trp	His	Cys	Gln	Pro	Leu	Gln	Thr	Tyr	Pro	Ser	Ser	Ser
305					310					315					320
Gly	Pro	Val	Glu	Val	Phe	Ile	Thr	Glu	Thr	Pro	Ser	Gln	Pro	Asn	Ser
				325					330					335	
His	Pro	Ile	Gln	Trp	Leu	Asp	Asp	Asp	Asp	Lys	Ala	Ala	Gly	Ser	Ile
			340					345					350		
Thr	Thr	Leu	Pro	Ala	Leu	Pro	Glu	Asp	Gly	Gly	Ser	Gly	Ala	Phe	Pro
		355					360					365			
Pro	Gly	His	Phe	Lys	Asp	Pro	Lys	Arg	Leu	Tyr	Cys	Lys	Asn	Gly	Gly
	370					375					380				
Phe	Phe	Leu	Arg	Ile	His	Pro	Asp	Gly	Arg	Val	Asp	Gly	Val	Arg	Glu
385					390				395						400
Lys	Ser	Asp	Pro	His	Ile	Lys	Leu	Gln	Leu	Gln	Ala	Glu	Glu	Arg	Gly
			405					410					415		
Val	Val	Ser	Ile	Lys	Gly	Val	Cys	Ala	Asn	Arg	Tyr	Leu	Ala	Met	Lys
			420					425				430			
Glu	Asp	Gly	Arg	Leu	Leu	Ala	Ser	Lys	Cys	Val	Thr	Asp	Glu	Cys	Phe
	435						440					445			

Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg

450

455

460

Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys

465

470

475

480

Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro

485

490

495

Met Ser Ala Lys Ser

500

<210> 5

<211> 400

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Hybrid

Polypeptide of Human Fibronectin Collagen-Binding

Domain and Human Epidermal Growth Factor

<220>

<221> INIT __MET

<222> (1)

<220>

<221> DOMAIN

<222> (2)..(341)

<223> /note="human fibronectin collagen-binding domain"

<220>

<221> PEPTIDE

<222> (343)..(347)

<223> /note=" enterokinase recognition sequence"

<220>

<221> PEPTIDE

<222> (348)..(400)

<223> /note="human epidermal growth factor"

<400> 5

Met Ala Ala Val Tyr Gln Pro Gln Pro His Pro Gln Pro Pro Pro Tyr

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Gly His Cys Val Thr Asp Ser Gly Val Val Tyr Ser Val Gly Met Gln

20 25 30

Trp Leu Lys Thr Gln Gly Asn Lys Gln Met Leu Cys Thr Cys Leu Gly

35 40 45

Asn Gly Val Ser Cys Gln Glu Thr Ala Val Thr Gln Thr Tyr Gly Gly

50 55 60

Asn Ser Asn Gly Glu Pro Cys Val Leu Pro Phe Thr Tyr Asn Gly Arg

65 70 75 80

Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg Gln Asp Gly His Leu Trp

85 90 95

Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp Gln Lys Tyr Ser Phe Cys

100 105 110

Thr Asp His Thr Val Leu Val Gln Thr Arg Gly Gly Asn Ser Asn Gly

115 120 125

Ala Leu Cys His Phe Pro Phe Leu Tyr Asn Asn His Asn Tyr Thr Asp

130 135 140

Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys Gly Thr Thr

145 150 155 160

Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly Phe Cys Pro Met Ala Ala

165 170 175

His Glu Glu Ile Cys Thr Thr Asn Glu Gly Val Met Tyr Arg Ile Gly
 180 185 190
 Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met Arg Cys Thr
 195 200 205
 Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Ile Ala Tyr Ser Gln
 210 215 220
 Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn Asp
 225 230 235 240
 Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr Cys
 245 250 255
 Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val Asp Gln Cys
 260 265 270
 Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp Ser Trp Glu
 275 280 285
 Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr Gly Arg Gly
 290 295 300
 Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser Ser
 305 310 315 320
 Gly Pro Val Glu Val Phe Ile Thr Glu Thr Pro Ser Gln Pro Asn Ser
 325 330 335
 His Pro Ile Gln Trp Leu Asp Asp Asp Asp Lys Asn Ser Asp Ser Glu
 340 345 350
 Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His Asp Gly Val Cys Met
 355 360 365
 Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn Cys Val Val Gly Tyr
 370 375 380
 Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys Trp Trp Glu Leu Arg

385 390 395 400

<210> 6

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR Sense
Primer for Human Fibronectin Collagen-Binding
Domain

<400> 6

gaggtaccat ggtacatatg gcagctgttt accaaccgca gcctcaccc 49

<210> 7

<211> 46

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR Antisense
Primer for Human Fibronectin Collagen-Binding
Domain

<220>

<400> 7

cgggatacctt actcgagcca ctggatgggg tgggagttgg gctgac 46

<210> 8

<211> 1053

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Modified Human
Fibronectin Collagen-Binding Domain

<220>

<221> conflict

<222> (109)

<220>

<221> conflict

<222> (206)

<220>

<221> conflict

<222> (270)

<220>

<221> conflict

<222> (374)

<220>

<221> conflict

<222> (681)

<400> 8

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tatggccact gtgtcacaga cagtgggtgtg gtctactctg tggggatgca gtggctgaag 120
acacaaggaa ataagcaa at gctttgcacg tgccctgggca acggagtcag ctgccaagag 180
acagctgtaa cccagactta cgggtggcaac tcaaatggag agccatgtgt cttaccattc 240
acctacaatg gcaggacgtt ctactcctgc accacagaag ggcgacagga cggacatctt 300
tggtgcagca caacttcgaa ttatgagcag gaccagaaat actctttctg cacagaccac 360
actgttttgg ttcagactcg aggaggaaat tccaatgggt ccttgtgcca cttccccttc 420
ctatacaaca accacaatta cactgattgc acttctgagg gcagaagaga caacatgaag 480
tggtgtggga ccacacagaa ctatgatgcc gaccagaagt ttgggttctg ccccatggct 540

31

<210> 11

<211> 489

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Human Basic

Fibroblast Growth Factor with Enterokinase

Recognition Sequence

<220>

<221> mutation

<222> (228)

<223> /note="mutation caused by polymerase chain
reaction"

<400> 11

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ggcgggcagcg ggcgccttccc gcccgggccac ttcaaggacc ccaagcggct gtactgcaaa 120
aacggggggct tcttctctgcg catccacccc gacggccgag ttgacgggggt ccggggagaag 180
agcgaccctc acatcaagct acaacttcaa gcagaagaga gaggagtcgt gtctatcaaa 240
ggagtgtgtg ctaaccgtta cctggctatg aaggaagatg gaagattact ggcttctaaa 300
tgtgttacgg atgagtgttt cttttttgaa cgattggaat ctaataacta caatacttac 360
cggatcaagga aatacaccag ttggtatgtg gcactgaaac gaactgggca gtataaactt 420
ggatcaaaaa caggacctgg gcagaaagct atactttttc ttccaatgtc tgctaagagc 480
tgagaattc
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489

<210> 12

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR Sense

Primer for Human Epidermal Growth Factor

<400> 12

gtgtcgacga cgatgataag aatagtgtact ctgaatgtcc cctg 44

<210> 13

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR Antisense

Primer for Human Epidermal Growth Factor

<400> 13

gaattcttag cgcagttccc accacttcag 30

<210> 14

<211> 186

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Human Epidermal

Growth Factor with Enterokinase Recognition

Sequence

<400> 14

gtcgacgacg atgataagaa tagtgactct gaatgtcccc tgtcccacga tgggtactgc 60
ctccatgatg gtgtgtgcat gtatatggaa gcattggaca agtatgcatg caactgtgtt 120
gttggtctaca tcggggagcg atgtcagtag cgagacctga agtgggtggga actgcgctaa 180
gaattc 186

<210> 15

<211> 1527

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Hybrid

Polypeptide of Human Fibronectin Collagen-Binding

Domain and Human Fibroblast Growth Factor

<400> 15

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 tatggccact gtgtcacaga cagtgggtgtg gtctactctg tggggatgca gtggctgaag 120
 acacaaggaa ataagcaa atgctttgcacg tgcctgggca acggagtcag ctgccaaagag 180
 acagctgtaa cccagactta cgggtggcaac tcaa atggag agccatgtgt cttaccattc 240
 acctacaatg gcaggacgtt ctactcctgc accacagaag ggcgacagga cggacatctt 300
 tgggtgcagca caacttcgaa ttatgagcag gaccagaaat actctttctg cacagaccac 360
 actgttttgg ttcagactcg aggaggaaat tccaatgggtg ccttgtgcca cttccccttc 420
 ctatacaaca accacaatta cactgattgc acttctgagg gcagaagaga caacatgaag 480
 tgggtgtggga ccacacagaa ctatgatgcc gaccagaagt ttgggttctg ccccatgggt 540
 gcccacgagg aaatctgcac aaccaatgaa ggggtcatgt accgcattgg agatcagtgg 600
 gataagcagc atgacatggg tcacatgatg aggtgcacgt gtgttgggaa tggctgtggg 660
 gaatggacat gcattgccta ctgcagctt cgagatcagt gcattgttga tgacatcact 720
 tacaatgtga acgacacatt ccacaagcgt catgaagagg ggcacatgct gaactgtaca 780
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 taccagtgtc actgctatgg ccgtggcatt ggggagtggc attgccaacc ttacagacc 960
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cccgccttgc ccgaggatgg cggcagcggc gccttcccgc cgggccactt caaggacccc 1140
 aagcggctgt actgcaaaaa cgggggcttc ttctgcgca tccaccccga cggccgagtt 1200
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 ggagtcgtgt ctatcaaagg agtgtgtgct aaccgttacc tggctatgaa ggaagatgga 1320
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 aataactaca atacttaccg gtcaaggaaa tacaccagtt ggtatgtggc actgaaacga 1440
 actgggcagt ataaacttgg atccaaaaca ggacctgggc agaaagctat actttttctt 1500
 ccaatgtctg ctaagagctg agaattc 1527

<210> 16

<211> 1224

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Hybrid

Polypeptide of Human Fibronectin Collagen-Binding

Domain and Human Epidermal growth factor

<400> 16

ggtaccatgg tacatatggc agctgtttac caaccgcagc ctcaccccca gcctcctccc 60
 tatggccact gtgtcacaga cagtgggtgtg gtctactctg tggggatgca gtggctgaag 120
 acacaaggaa ataagcaaatt gctttgcacg tgcctgggca acggagtcag ctgccaagag 180
 acagctgtaa cccagactta cgggtggcaac tcaaatggag agccatgtgt cttaccattc 240
 acctacaatg gcaggacgtt ctactcctgc accacagaag ggcgacagga cggacatctt 300
 tgggtgcagca caacttegaa ttatgagcag gaccagaaat actctttctg cacagaccac 360
 actgttttgg ttcagactcg aggaggaaat tccaatgggtg ccttgtgcca ctcccccttc 420
 ctatacaaca accacaatta cactgattgc acttctgagg gcagaagaga caacatgaag 480
 tgggtgtggga ccacacagaa ctatgatgcc gaccagaagt ttgggttctg ccccatggct 540
 gccacagagg aaatctgcac aaccaatgaa ggggtcatgt accgcattgg agatcagtgg 600

gataagcagc	atgacatggg	tcacatgatg	agggtgcacgt	gtgttgggaa	tggtcgtggg	660
gaatggacat	gcattgccta	ctcgcagctt	cgagatcagt	gcattgttga	tgacatcact	720
tacaatgtga	acgacacatt	ccacaagcgt	catgaagagg	ggcacatgct	gaactgtaca	780
tgcttcggtc	agggctcgggg	cagggtggaag	tgtgatcccg	tcgaccaatg	ccaggattca	840
gagactggga	cgttttatca	aattggagat	tcatggggaga	agtatgtgca	tggtgtcaga	900
taccagtgct	actgctatgg	ccgtggcatt	ggggagtggc	attgccaaacc	tttacagacc	960
tatccaagct	caagtgggcc	tgtcgaagta	tttatcactg	agactccgag	tcagcccaac	1020
tcccacccca	tccagtggct	cgacgacgat	gataagaata	gtgactctga	atgtcccctg	1080
tcccacgatg	ggtactgcct	ccatgatggg	gtgtgcatgt	atattgaagc	attggacaag	1140
tatgcatgca	actgtgttgt	tggctacatc	ggggagcgat	gtcagtaccg	agacctgaag	1200
tggtggggaac	tgcgctaaga	attc				1224